

CASE WW/3-22352/A/PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE PCT NATIONAL STAGE APPLICATION OF  
JONATHAN HUGHES ET AL.

Group Art Unit: 1651

Examiner: Ariani, Kade

INTERNATIONAL APPLICATION NO. PCT/EP 05/000520

FILED: January 20, 2005

FOR: PRODUCTION OF A FERMENTATION  
PRODUCT

U.S. APPLICATION NO: 10/587,583

35 USC 371 DATE: JULY 28, 2006

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

APPEAL BRIEF

Sir:

This Appeal is from the Final Rejection mailed from the PTO on October 27, 2009.

A Notice of Appeal was filed electronically on January 29, 2010.

Filing of this Appeal Brief is timely up to and including March 29, 2010. The Brief is accompanied by the requisite fee under 41.20.

The Commissioner is authorized to charge any fee due, or credit any overcharge, as a result of this Amendment to Deposit Account No. 03-1935.

(1) REAL PARTY OF INTEREST

The real party of interest, by virtue of an assignment recorded in the United States Patent and Trademark Office on May 10, 2007 reel/frame 019301/0447 is:

Ciba Specialty Chemicals Water Treatments Ltd.  
P.O. Box 38  
Cleckheaton Road  
Low Moor,  
Bradford, West Yorkshire  
BD12 0JZ, England

(2) RELATED APPEALS AND INTERFERENCES

Appellants are not aware of any related appeals and interferences for the above application.

(3) STATUS OF THE CLAIMS

Claims 1-3, 5-21 and 23 are pending in the application.

Claims 1-3, 5-21 and 23 are rejected.

(4) STATUS OF AMENDMENTS

The claims were last amended on February 5, 2009. These amendments were entered.

This brings up to date the status of the claims. A clean copy of the claims is attached in the (8) Claims Appendix.

(5) SUMMARY OF THE CLAIMED SUBJECT MATTER

Claim 1 is the only independent claim and is directed to a process of separating suspended solids from a fermentation liquor by subjecting the liquor to a solids-liquid separation stage. The fermentation liquor is defined as being produced in a fermentation process for the production of a fermentation product. See page 5, lines 21-25. The fermentation liquor has been subjected to a temperature of at least 50°C. See page 5, line 26.

The term "fermentation liquor" is used to include mixtures often referred to as "mixed fermentation liquor" or "fermentation broth". These liquors include those resulting from agricultural plant derived materials that have been subjected to one or more fermentation stages. See page 5, lines 15-20.

The solids-liquid separation stage is assisted by a treatment system, which system comprises an anionic polymer selected from natural polymers and modified natural polymers having an anionic charge such that the equivalent weight is below 300, and synthetic polymers formed from at least 50% by weight anionic monomer units which anionic monomer units are selected from the group consisting of (meth) acrylic acid or salts, maleic acid or salts, itaconic acid or salts and fumaric acid or salts.

Support for the above claim may be found at the bottom of page 5 and first few lines of page 6. As to support for the at least 50% by weight anionic monomer units may be found on page 7, lines 25-27. Support is further given in the examples pages 15 thru 18. Various anionic polymers (almost all of which are at least 50 % by weight anionic monomer units) are tested on a post-distillation heat treated fermentation liquor to establish filterability or dewatering efficiency. See page 15, lines 1-5 and tables 1 and 2 on page 16 and table 3 on page 17.

The anionic polymer may be a synthetic, modified natural (semi-natural) or natural polymer. See page 7, lines 30-31. The synthetic anionic polymer is formed from the group of anionic monomers selected from the group consisting of (meth)acrylic acid or salts, maleic acid or salts, itaconic acid or salts and fumaric acid or salts. See page 8, lines 8-10.

The appellants have unexpectedly found that the yield of process can be improved by effecting a rapid but efficient solids-liquid separation of the solid residues from a fermentation liquor that has been subjected to elevated temperatures. The process enables the recovery of soluble components from the distillation column still bottom liquors because they are rendered insoluble as a result of the heat treatment, or exposure to a distillation process for the production of a fermentation product. See page 6, lines 12-15.

Thus the process is especially suited to the dewatering of heat treated fermentation liquor and separation of solids therefrom. See page 6, lines 21-22.

#### (6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

## **Double Patenting Rejections**

There are several double patenting rejections outstanding. These rejections are not to be reviewed on appeal. Appellents will file terminal disclaimers to overcome the double patenting rejections once the 35 USC 103(a) rejection below is overcome.

### **35 USC § 103 (a)**

**Claims 1-3, 5-21 and 23 are rejected under 35 USC 103(a) as being unpatentable over Verser, US 6,927,048 in view of Coffey, US 2003/0155091 and further in view of Ovenden and Song.**

## **(7) ARGUMENTS**

The Appeal will stand or fall on the basis of claim 1.

### **Basis of the Examiner's Rejection**

#### **35 USC § 103 (a)**

**Claims 1-3, 5-21 and 23 are rejected under 35 USC 103(a) as being unpatentable over Verser, US 6,927,048 in view of Coffey, US 2003/0155091 and further in view of Ovenden and Song.**

Verser teaches a fermentation liquor produced in a fermentation process for the production of a fermentation product. Verser further teaches the ethanol is removed from the water stream which water stream is discharged from the column and separated by a simple liquid-solid separation such as centrifugation or filtration, into the solid base for recycle.

Examiner agrees that Verser does not teach the treatment system comprises an anionic polymer as presently claimed.

Examiner uses Coffey to teach that dewatering may be done by the use of polymeric flocculants which comprise cationic and anionic monomers. Coffey further teaches that using such polymers for displacing unwanted soluble or colloidal materials from an aqueous cellulosic suspension increases the efficiency of dewatering.

Appellants point out that Coffey is directed to cellulosic suspensions encountered in papermaking. A papermaking suspension is not a fermentation liquor.

While Coffey does teach polymers formed from about 0.1 to 9.9 mole percent of one or more cationic or anionic monomers and from 0.1 to about 49.9 mole percent of one or more zwitterionic monomers, there is absolutely no suggestion within the bounds of Coffey to form a synthetic polymer encompassed by the present claim limitations. That is, Coffey does not teach a polymer formed from at least 50% by weight anionic monomer units which anionic monomer units are selected from the group consisting of (meth)acrylic acid or salts, maleic acid or salts, itaconic acid or salts and fumaric acid or salts. Coffey teaches only that the polymer be 0.1 to 9.9 mole percent of one or more cationic or anionic monomers. A 50 wt. % anionic monomer content is far outside the teachings of Coffey.

Thus even if Coffey were to be combined with Verser, and there was some motivation to combine (appellants strongly object that there is any motivation to combine), one would not arrive at the present claim limitations.

Further, the appellants question the motivation to even look to Coffey as a resource to call upon to improve the process of separating suspended solids from a fermentation liquor as Coffey is directed to cellulosic suspensions in papermaking. A papermaking slurry is generally less than 1 percent dry weight of the solids in the slurry. The solids are primarily cellulosic. See Coffey page 1, paragraph [0002].

A fermentation liquor frequently contains biomass derived from materials containing hemicelluloses and lignocellulosic compounds in which case the fermentation liquor may contain lignin and lignin-type substances or alternatively may be derived from purer carbohydrate substrates such as sugars and crops producing starch. See page 6, lines 20-25. The weight percent dry matter of the fermentation liquor is frequently in the range of 7%, a far greater concentration than that for cellulosic slurries for making paper. See present example 1, page 15, last line of page.

Examiner has additionally rely on two separate references Song and Ovendon to teach anionic polymers as presently claimed, that is anionic polymers having an anionic charge such that the equivalent weight is below 300 and the synthetic polymer is formed for at least 50% by weight anionic monomer units.

Examiner points out that Song teaches a charged polyacrylamide will be more effective as flocculant. See col. 1, lines 10-12.

Examiner goes on to say that a person skilled in the art would have recognized that charge density and intrinsic viscosity of the charged polymer were result effective variables and could have been optimized. See page 7, last paragraph of examiners response of October 27, 2010.

Appellants submit that while it is well known to vary monomer ratio of a polymer, the examiner's references have given no guidance as to the anionic monomer weight percent.

While Song teaches that a charged polyacrylamide will be more effective as a flocculant, Song teaches that the charged polyacrylamides are **cationic**. Song makes no statements which would teach or suggest the use of an **anionic** polyacrylamide as a flocculant. The purpose of Song is to explore cationic copolymers of acrylamide and acryloxyloxyethyl dimethylbenzyl ammonium chloride. See Introduction and abstract of Song.

Examiner further relies on Ovenden who allegedly teaches improved flocculation efficiency using the synergy between cationic polymer (CM) and anionic polymer (Abstract). Examiner points to the table at the bottom of page 226, as teaching anionic polyacrylamide of various charge densities.

Appellants point out that Ovenden's alleged cationic polymer (CM) is not a polymer at all but inorganic cationic microparticles. While Ovenden teaches anionic polyacrylamide as a flocculant in solids removal in waste water treatment and in fine solids retention in papermaking processes, Ovenden's description of the anionic polyacrylamide as medium anionic or low anionic does not suggest what weight % of the polyacrylamide is formed from anionic monomer units nor does it suggest which anionic monomers are used to form the anionic polyacrylamide.

Thus the combination of Verser with Coffey and further in view of Song and Ovenden does not suggest the present claim limitations. The rejection is completely deficient in regard to the use of an anionic polymer formed from at least 50 wt. % anionic monomer units which anionic monomer units are selected from the group consisting of (meth)acrylic acid or salts, maleic acid or salts, itaconic acid or salts and fumaric acid or salts.

Appellants further believes that it is important to point out that while it is well known to vary the monomer ratio of a polymer, the examiner's references provide no guidance as to what that ratio should be and under what circumstances a particular ratio is effective. The references relied on do not teach with any specificity the presently claimed invention even when combined.

Some comments are necessary by the appellants to address the examiner's statement that "the charge density and the molecular weight of an anionic polymer or cationic polymer can be varied and that a person skilled in the art at the time the invention was made would have recognized that the charge density and intrinsic viscosity of the charged polymer (cationic and anionic) were result effective variables and could have been optimized".

This statement trivializes almost all inventions relating to the use of particular polymers for specific purposes. Because one skilled in the art recognizes that polymers may be varied in many different ways (Mw, Mn, charge, crosslinking etc.), does **not** render obvious the use of a particular polymer for a specific use. **In other words because one skilled in the art knows how to make many different kinds of polymers does not in any way provide a solution to which polymer should be used in which application.**

Additionally appellants direct the Board to Table 3 on page 17 of the present disclosure. The Table 3 shows clearly the use polymers of at least 50 wt. % anionic content, a very significant improvement in dewatering efficiency is observed in fermentation liquors. This effect holds true regardless of the intrinsic viscosity of the polymer or the specific anionic polymer or copolymer. Those polymers of less than 50 wt. % anionic content are clearly inferior. The additional Tables 1 and 2 on page 16 show the same trend. A variety of anionic polymer or copolymers are tested of a range of intrinsic viscosities. For example, sodium acrylate:acrylamide, poly-2-acrylamido-2-methylsulphonate:acryalmide and polysodium acrylate which range from 10 to 100 anionic wt. % and ranging from an intrinsic viscosity from 1 to 27 dl/g are tested. The trend is clear. Unexpected improved performance in dewatering efficiency is shown by all anionic polymers or copolymers for a wide range of intrinsic viscosities as long as the polymer or copolymer is at least 50 wt. % anionic monomer units.

This improvement could not have been derived from the references cited.

#### **Two References Attached**

Applicants enclose two references which describe PERCOL E24 (See page 407, 2<sup>nd</sup> col., section 2.1) and PERCOL 173 (See submitted MSDS, second page under Chemical Family). Both documents are published later than the priority date of the present application but should be admissible for clarifying the specific makeup of the two anionic polymers mentioned in Ovenden (table 1).

PERCOL E24 is an anionic polyacrylamide-based copolymer having a content of 15 % polyacrylate units.

PERCOL 173 is anionic polyacrylamide-base copolymer having a content of 5% of polyacrylate units.

**In summary, the Appellants believe the combination of references to not make obvious the present claim limitations for the following reasons:**

- While Verser is directed to a fermentation process for the production of a fermentation product and further teaches that ethanol is removed from the water stream which water stream is discharged from the column and separated by a simple liquid-solid separation such as centrifugation or filtration, into the solid base for recycle, there is no teaching or motivation within Verser to use an anionic polymer for effecting the liquid-solid separation.
- Even with the combination of Coffey, the anionic content limitation of the polymer used in the presently claimed method is not suggested, as Coffey teaches a maximum of about 9.9 mole %.
- Furthermore, the two references, Verser and Coffey's, are directed to solid-liquid separation of quite different substrate. Coffey is directed to cellulosic suspensions for papermaking while Verser is directed to solid-liquid separations of fermentation liquors. There is nothing really linking the two references other than they are both directed to solid-liquid separations. One skilled in the art knows that what works for one substrate may not be suitable for another. Thus there can be little expectation of success in using the polymers defined in Coffey for cellulosic or pulp suspension in previously heated fermentation liquors of Verser.
- Even if there were a reason one skilled in the art looking at Verser would be directed to Coffey, one skilled in the art would be directed to a maximum of 9.9 anionic monomer content by Coffey.

- The reliance on Song and Ovendun by the Office is especially unreasonable. While Song teaches that a charged polyacrylamide will be more effective as a flocculant, Song teaches that the charged polyacrylamides are **cationic** and makes no statements which would teach or suggest the use of an **anionic** polyacrylamide as a flocculant. Furthermore, Song teaches no link between the use of his charged cationic polyacrylamides and fermentation liquors. And even if there were a link to fermentation liquors, this reference would still not work as the reference fails to teach the use of a synthetic polymer of at least 50 % by weight anionic monomer units.
- The Office further relies on Ovenden who allegedly teaches improved flocculation efficiency using the synergy between cationic polymer (CM) and anionic polymer (Abstract). Examiner points to the table at the bottom of page 226, as teaching anionic polyacrylamide of various charge densities. Two anionic polyacrylamides are listed in this table. They are PERCOL® E24 and PERCOL® 173. Applicants have attached an MSDS for PERCOL® E24 and a description from the literature of PERCOL® 173. These documents are supplied as evidence that the anionic polyacrylamides used by Ovenden do not meet the present claim limitations, that is these polymers are not formed from at least 50 % by weight anionic monomer units.
- Thus, Ovenden's description of the anionic polyacrylamide as medium anionic or low anionic does not suggest what weight % of the polyacrylamide is formed from anionic monomer units nor does it suggest which anionic monomers are used to form the anionic polyacrylamide.
- The Office believes appellants are arguing the references individually, but as explained above even if the references are combined they do not arrive at the present claim limitations. In particular, all the references are deficient as to suggesting or teaching a polymer of at least 50 percent by weight anionic monomer content.
- Examiner is also of the opinion that the references of Song and Ovenden teach that a person skilled in the art would have recognized that charge density and intrinsic viscosity of the charged polymer were result effective variables and could have been optimized.
- This opinion trivializes almost all inventions relating to the use of particular polymers for specific purposes. Because one skilled in the art recognizes that polymers may be varied in many different ways (Mw, Mn, charge, crosslinking etc.), does **not** render obvious the use of a

particular polymer for a specific use. In other words because one skilled in the art knows how to make many different kinds of polymers does not in any way provide a solution to which polymer should be used in which application.

- Examiner has assembled a grouping of four references, only one of which even discusses a fermentation process. Three of the references are primarily directed to papermaking processes. A key claim limitation is not suggested by any of the references. It is unclear why one skilled in the art of fermentation liquors would refer to any of the secondary references primarily directed to papermaking. And then even if there was a motivation to look at these references, there is no direction to select a polymer of greater than 50 percent by weight anionic monomer unit content.
- And finally, the present examples show the criticality of using a polymer of at least 50 percent by weight anionic monomer unit content to improve dewatering efficiency. Clearly there is nothing in any of the references which would lead one skilled in the art to suppose that such an improvement could have been accomplished by using a polymer with at least 50 percent by weight anionic monomer unit content.

In light of the above discussions, Appellants respectfully submit that the rejections of claims 1-3, 5-21 and 23 have been rebutted and respectfully ask that the rejections be reconsidered and reversed.

Respectfully submitted,



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(8) CLAIMS APPENDIX

1. (previously presented): A process of separating suspended solids from a fermentation liquor by subjecting the liquor to a solids-liquid separation stage, wherein the fermentation liquor is produced in a fermentation process for the production of a fermentation product, in which the fermentation liquor has been subjected to a temperature of at least 50°C, wherein the solids-liquid separation stage is assisted by a treatment system, characterised in that the treatment system comprises an anionic polymer selected from natural polymers and modified natural polymers having an anionic charge such that the equivalent weight is below 300, and synthetic polymers formed from at least 50% by weight anionic monomer units which anionic monomer units are selected from the group consisting of (meth) acrylic acid or salts, maleic acid or salts, itaconic acid or salts and fumaric acid or salts.

2. (original): A process according to claim 1 in which the fermentation liquor is subjected to a distillation stage in which the fermentation product is recovered, wherein the liquor is removed from the distillation stage as a stillage stream and then subjected to the solids-liquid separation stage.

3.(previously presented): A process according to claim 1 in which the treatment system comprises an anionic polymer formed from at least 65% by weight anionic monomer units.

4. (cancelled).

5. (previously presented): A process according to claim 1 in which the anionic polymer exhibits an intrinsic viscosity of at least 4 dl/g (measured using a suspended level viscometer in 1M NaCl buffered to pH 7.5 at 25°C).

6. (previously presented): A process according to claim 1 in which the treatment system further comprises addition of a cationic polymer that exhibits an intrinsic viscosity below 4dl/g (measured using a number 1 suspended level viscometer in 1M NaCl buffered to pH 7.0 at 25°C).

7. (original): A process according to claim 6 in which the cationic polymer exhibits a charge density of at least 3 meq/g.

8. (previously presented): A process according to claim 6 in which the cationic polymer is selected from the group consisting of polyamines, amine/epihalohydrin addition polymers, polymers of dicyandiamide with formaldehyde, polymers of diallyldimethyl ammonium chloride (DADMAC), cationic starch and cationic inulin, polymers of dialkyl amino alkyl (meth) acrylates (or salts) and dialkyl amino alkyl (meth) acrylamides (or salts).

9. (previously presented): A process according to claim 6 in which the anionic polymer and cationic polymer are added sequentially.

10. (previously presented): In a process according to claim 1 in which the dose of anionic polymer is at least 50 grams per tonne (based on dry weight of fermentation liquor).

11. (previously presented): A process according to claim 6 in which the dose of cationic polymer is at least 50 grams per tonne (based on dry weight of fermentation liquor).

12. (previously presented): A process according to claim 1 in which the treatment system further comprises addition of a siliceous material.

13. (original): A process according to claim 12 in which the siliceous material is selected from the group consisting of silica based particles, silica microgels, colloidal silica, silica sols, silica gels, polysilicates, cationic silica, aluminosilicates, polyaluminosilicates, borosilicates, polyborosilicates, zeolites and swellable clays.

14. (previously presented): A process according to claim 12 in which the siliceous material is an anionic microparticulate material.

15. (previously presented): A process according to claim 12 in which the siliceous material is a bentonite type clay.

16. (previously presented): A process according to claim 12 in which the siliceous material is selected from the group consisting of hectorite, smectites, montmorillonites, nontronites, saponite, saucomite, hormites, attapulgites and sepiolites.

17. (previously presented): A process according to claim 1 in which the fermentation liquor is subjected to a mechanical dewatering stage during or subsequent to application of the treatment system.

18. (original): A process according to claim 17 in which the mechanical dewatering step is selected from the group consisting of a centrifuge, a screw press, a filter press, a belt filter press a horizontal belt filter, and a pressure filter.

19. (previously presented): A process according to claim 1 in which the treated liquor from which suspended solids have been removed are recycled and used as wash water.

20. (previously presented): A process according to claim 1 in which the fermentation liquor comprises lignin and in which the separated solids are dewatered and then subjected to a drying stage to provide a dry solid material and in which the dry solid material is used as a solid fuel.

21.(previously presented):A process according to claim 1 in which the fermentation liquor is derived from crop sugars and in which the separated solids are dewatered and then subjected to a drying stage to provide a dry solid material and in which the dry solid material is used as a solid fuel or as an animal feed.

22. (cancelled).

23. (previously presented): a process according to claim 1 in which the fermentation product is selected from the group consisting of ethanol, glycerol, acetone, n-butanol, butanediol, isopropanol, butyric acid, methane, citric acid, fumaric acid, lactic acid, propionic acid, succinic acid, itaconic acid, acetic acid, acetaldehyde and 3-hydroxypropionic acid, glycolic acid, tartaric acid, and amino acids or salts of any of these acids.

(9) EVIDENCE APPENDIX

MSDS sheet for PERCOL E24 and Huining, Xian, et al., *J. of Colloid and Interface Science* 283,(2005), 406-413.



Engineered Drilling Solutions Inc.

# Engineered Drilling Solutions Inc.

## MATERIAL SAFETY DATA SHEETS

Effective October 01, 2006



Engineered Drilling Solutions Inc.

## SECTION 1 – IDENTIFICATION OF PRODUCT

**Product Name:** Percol E24

**Chemical Family:** Copolymer of acrylamide and sodium acrylate

**Product Use:** Drilling Mud Additive

<b>Work Place Hazardous Information Systems Data (WHMIS)</b>	<b>Transportation Of Dangerous Goods (TDG)</b>
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WHMIS Classification: Not a controlled product  
Work Place Hazard: N / A

TDG Classification: Not a regulated product  
Package Group: N / A  
UN Number (PIN): N / A

## SECTION 2 – HAZARDOUS INGREDIENTS

No Hazardous Ingredients

## SECTION 3 – PHYSICAL DATA

**Physical State:** Solid

**Odour and Appearance:** White granular solid, little odour

**Odour Threshold:** N / A

**Vapor Pressure:** N / A

**Vapor Density:** N / A

**Evaporation Rate:** N / A

**Coefficient of Water To Oil Distribution:** N / A

**Boiling Point:** N / A

**Freezing Point:** N / A

**Melting Point:** N / A

**pH:** 5.5 - 6.5 in a 1 % solution

**Solubility:** Soluble

**Specific Gravity:** N / A

**Percent Volatile by Volume:** N / A



Engineered Drilling Solutions Inc.

## SECTION 4 – FIRE AND EXPLOSION DATA

**Conditions Of Flammability:** Very low risk

**Flashpoint:** N / A

**Lower Flammable Limit:** N / A

**Upper Flammable Limit:** N / A

**Auto Ignition Temperature:** N / A

### Means Of Extinction

Dry Chemical, Carbon Dioxide, Foam

### Hazardous Combustion Products

None known

### Explosion Data

As with most organic powders, flammable dust clouds may be formed; avoid generating dust.

Avoid sources of ignition

### Special Firefighting Procedures

Firefighters must wear appropriate breathing apparatus and clothing. Water may cause slipperiness

## SECTION 5 – REACTIVITY DATA

**Chemically Stable?:** Yes

### Conditions Of Chemical Instability

**Incompatible With Other Substances?:** Yes

### Incompatible Substances

Avoid strong oxidizing agents and caustic solutions

### Conditions Of Reactivity

None known

### Hazardous Decomposition Products

None known

### Hazardous Polymerization:

Will not occur

**Percol E24**



Engineered Drilling Solutions Inc.

## SECTION 6 – TOXICOLOGICAL PROPERTIES

	Skin	Skin Absorption	Eye Contact	Inhalation	Ingestion
<b>Routes Of Entry:</b>	Yes	Yes	Yes	Yes	Yes
<b>Irritation:</b>	Prolonged exposure may cause irritation, swelling, or dermatitis		Exposure may cause irritation to eyes and eye lids	May cause slight irritation of nose and throat	May cause nausea and vomiting

### General Irritancy Of Product

### Exposure Limits

(8 hour TWA, total inhalable dust) ACGIH: 10 mg/m cubed; OSHA PEL: 10mg/m cubed; MFRS: recommendation: 10 mg/m cubed

**Effects Of Acute Exposure:** None known  
**Effects Of Chronic Exposure:** None known  
**Sensitization To Product:** Not a sensitizer  
**Carcinogenicity:** N / A  
**Teratogenicity:** N / A  
**Reproductive Toxicity:** N / A  
**Mutagenicity:** N / A  
**Synergistic Products:** N / A

## SECTION 7 – PREVENTIVE MEASURES

### Gloves: See Clothing

**Respirator:** If using in a confined non ventilated space, use of an approved dust mask is advised

### Eye: See Clothing

**Clothing:** Chemical resistant clothing is recommended including gloves, apron, and goggles  
**Other:**

### Engineering Controls

### Storage and Handling Requirements

Avoid inhalation of vapour and mist. Avoid prolonged skin contact. Use with appropriate ventilation. Avoid high temperatures, ingestion, dusty conditions, and all sources of ignition. Wet material may cause slippery conditions.

### Leak And Spill Procedures

Wear protective equipment. Recover material into approved containers. Do not allow into water ways. Wash spill site after cleanup.

**Percol E24**



Engineered Drilling Solutions Inc.

#### **Waste Disposal**

All waste should be disposed of according to Federal, Provincial, and Local Regulations. Containers should NOT be reused. Containers should be disposed of in accordance with government regulations

### **SECTION 8 – FIRST AID MEASURES / OTHER HAZARDOUS INFORMATION**

#### **Specific Measures**

##### **Skin First Aid**

Wash with soap and water. If adverse symptoms develop, seek medical attention

##### **Eye First Aid**

Flush eyes with running water for at least 15 minutes. If adverse symptoms develop, seek medical attention

##### **Inhalation First Aid**

Move victim to fresh air. If breathing has stopped, apply artificial respiration. If breathing is difficult, administer Oxygen. Seek immediate medical attention.

##### **Ingestion First Aid**

Do not induce vomiting. Give large quantities of water, if conscious. Call a physician

### **SECTION 9 – PREPARATION INFORMATION**

**Prepared By:** Safety Department

**Phone:** (403) 234-7771 **Preparation Date:** October 01, 2006

*THE INFORMATION AND SUGGESTIONS CONTAINED HEREIN ARE GIVEN IN GOOD FAITH. NO WARRANTY, EXPRESSED OR IMPLIED, IS MADE.*



# Cationic-modified cyclodextrin nanosphere/anionic polymer as flocculation/sorption systems

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## Abstract

Simultaneous removal of dissolved and colloidal substances has been a challenging task. The cationic-modified  $\beta$ -cyclodextrin nanospheres synthesized in this work, in conjunction with a water-soluble polyacrylamide-based anionic polymer, potentially provide a novel approach to address the problem. The cyclodextrin was rendered cationic using (2,3-epoxypropyl)trimethylammonium chloride as a reagent. The cationicity of the modified cyclodextrin and the reaction between cyclodextrin and the reagent were characterized by electrophoresis measurement, polyelectrolyte titration, and NMR. As a dual-component flocculation system, the cationic cyclodextrin/anionic polymer significantly induced clay flocculation, lowering the relative turbidity of the clay suspension over a wide pH range. Meanwhile, as a nanospherical absorbent, the modified cyclodextrins exhibited strong affinity toward aromatic compounds via inclusion complex formation in the hydrophobic cavities, which was monitored by UV spectroscopy. These systems facilitated the simultaneous removal of dissolved and colloidal substances, which was unachievable previously. In addition, the interaction between anionic polymers and the clay particles pretreated with cationic cyclodextrin was investigated in order to reveal the flocculation mechanism.

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**Keywords:** Cationic cyclodextrin; Clay flocculation; Anionic polymer; Inclusion complex formation; Sorption

## 1. Introduction

Cyclodextrin, or cyclomaltoheptose, is a derivative of sugar produced from bacterial action on starch [1,2]. Among various cyclodextrins,  $\beta$ -cyclodextrin, consisting of seven glucose units, has been used extensively. The cyclodextrin structure takes the form of a toroid or a hollow tapering cone. The interior of the toroid, or the central cavity, is hydrophobic as a result of the electron-rich glycosidic oxygen atoms. The exterior of the toroid is hydrophilic, capable of interacting with aqueous medium during solvation. The unique architectural conformation of cyclodextrin is a result of the process of molecular rearrangement [3] and determines its specific chemical characteristic and behavior to the solvent environment [4].

A number of applications of cyclodextrin involve the formation of inclusion complexes in the liquid and solid phases [5–7]. Organic and inorganic molecules of appropriate size can be incorporated into the cyclodextrin cavity to form inclusion complexes. Hydrophobic molecules, or hydrophobic functional groups of molecules, can be included in the cyclodextrin cavity in the presence of water, if their molecular dimensions correspond to those of the cyclodextrin cavity. When the hydrophobic cavity of the cyclodextrin is filled partially or wholly with another molecule or substrate, an “inclusion complex” occurs. Several factors that influence the extent to which a guest can be incorporated in the central cavity of the cyclodextrin include steric effect, hydrophobicity, and various interactions involving van der Waals forces, dispersive forces, dipole–dipole interactions, electrostatic forces, and hydrogen bonding [3].

The interaction of water molecules and cyclodextrin is in fact a major concern, as most drug delivery systems

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are in aqueous systems. The moisture adsorption properties of modified cyclodextrins affect the physicochemical properties and stability of the formulated drug, especially those where side chains occur. Introduction of polymer chains (e.g., dendrimer poly(ethylenimine) or poly-*N*-isopropylacrylamide) to cyclodextrin has been widely applied to drug complexations [8–10]. Derivatives of cyclodextrin with specific functional groups have also been synthesized and found applications in drug targeting [11,12]. Modified cyclodextrins show better solubility and offered broader range of applications. Charge-bearing cyclodextrin has been reported as chiral additives in capillary electrophoresis [13,14]. The zeta potential of cyclodextrin nanospheres has been used to characterize the ionic properties of the modified cyclodextrins [15,16]. Ionic derivatives of cyclodextrin have been shown to form stronger complexes with drugs or chemicals that carry opposite charges.

In pulp production and papermaking, wood chemicals such as hemicelluloses, resin acids, and fatty acids are released during mechanical pulping operations. These lipophilic substances combine with the process water as dissolved and colloidal substances (DCS) bearing anionic charges [17]. DCS promote microbial growth and eventually cause deposition problems on the forming wire, thereby reducing water drainage [18]. Soluble substances also often coexist with colloidal substances in other types of industrial process water or wastewater. To solve this problem, several methods are employed to remove DCS in the paper slurry, especially for systems involving closed white water systems. The most common practice is the addition of cationic or nonionic polymers as flocculants to the system [19–22]. In this method, the DCS, particularly the colloidal substances, are removed via adsorption and formation of polyelectrolyte complexes. However, much attention has been paid to the removal of colloidal particles. Soluble contaminants, those with low molecular weight in particular, are unlikely to be removed by polyelectrolytes via complex formation. There is a lack of systems for simultaneous removal of soluble or dissolved and colloidal substances in water. Conventional adsorbents are effective in removing soluble parts but cannot form a dual-component system with polymer for the removal of colloidal particles via flocculation.

This work intended to synthesize a range of cationic cyclodextrin nanospheres aimed at removing soluble and colloidal substances in process water or wastewater simultaneously. The investigation was focused on the flocculation of filler clay particles using cationic cyclodextrin in conjunction with an anionic polyacrylamide-based polymer and the elimination of aromatic compounds in the clay suspension via the formation of inclusion complex with the cationic cyclodextrin. The interaction between cationic cyclodextrin and anionic polymers in inducing clay flocculation was also explored.

## 2. Experimental

### 2.1. Materials

$\beta$ -Cyclodextrin (99 wt%) obtained from Acros Organics was used as received. The cationic reagent (2,3-epoxypropyl)trimethylammonium chloride (71 wt%) (ETMAC) from Sigma-Aldrich was used as received. Polymers Percol 173 and E24, anionic polyacrylamide-based copolymers, were supplied by CIBA Chemicals and used as received. Percol 173 and Percol E24 have weight average  $M_w$  of about 12–14 million and the degrees of substitution are 5 and 15%, respectively. Fine filler clay was from Imerys, UK. The average diameter and the zeta potential of clay particles are about 3.0  $\mu\text{m}$  and  $-35.0\text{ mV}$ , respectively. Standard hydrochloric acid and sodium hydroxide solution were used to adjust pH. Toluene and benzoic acid from Sigma-Aldrich were used as received. The standard polyelectrolytes for colloidal titration, potassium polyvinyl sulfate (PVSK) ( $M_w$  170,000) and polyDADMAC and poly(diallyldimethyl ammonium chloride) (polyDADMAC) ( $M_w$  400,000–500,000, 20% in water) were purchased from Sigma-Aldrich. The water used was distilled and deionized.

### 2.2. Synthesis of cationic cyclodextrin

A range of cationic  $\beta$ -cyclodextrin-2-hydroxypropyltrimethylammonium chloride (HPTA- $\beta$ CD) was prepared using cationic reagent ETMAC. In a typical synthesis process, solutions of 0.4 wt%  $\beta$ -cyclodextrin in deionized and distilled water were first prepared and the pH was adjusted to 4.3. In a 100-ml flask equipped with reflux condenser, 0.6 ml of ETMAC was added to 50 ml of the cyclodextrin solution above. The mixture was subjected to continuous magnetic stirring for 2 h. The final solid product was collected after evaporation under vacuum.

The zeta potential of the modified cyclodextrin was determined according to the operating instructions of the DELSA 440SX. NMR spectroscopy was used to further identify the substitution of the cyclodextrin. About 0.05 g of the solid product was dissolved in 1 g of dimethyl sulfoxide (DMSO-d6) prior to  $^1\text{H}$  NMR analysis at 400 MHz using a UNITY 400 NMR spectrometer (Varian Associates, Inc., USA).

### 2.3. Characterization of clay flocculation

Clay flocculation was performed in a dynamic drainage jar (DDJ) setup using a photometric dispersion analyzer (Rank Brother, Inc., UK). Dynamic flocculation was conducted in the same manner as addressed elsewhere [23]. The clay suspension was circulated through the PDA using a peristaltic pump at a rate of 65 ml/min. The propeller speed in the DDJ was maintained at 500 rpm. The pH of the clay suspension was maintained at about 6, unless otherwise indicated. There was no electrolyte added to the suspension.

The cationic cyclodextrin was added prior to the anionic polymer.

#### 2.4. Determination of adsorption isotherms of anionic polymers

The interaction between clay particles and cationic cyclodextrin was investigated. Different volumes of the prepared cationic cyclodextrin stock solution were added to a mixture of 5 ml of 0.04 wt% clay. The mixture was thoroughly mixed and allowed to settle overnight at room temperature. It was then centrifuged at 3500 rpm for 15 min, and the precipitate was separated. The cationic cyclodextrin-treated clay particle (Ccy-clay) dispersion was then prepared with the addition of precipitated clay particles to 10 ml deionized and distilled water. To 5 ml of the Ccy-clay dispersion, 5 ml of 0.001 M NaCl was added. To maintain consistency of results among the samples, the pH of the sample mixtures was adjusted to approximately 4 by adding 0.05 vol% HCl solution prior to zeta potential analysis. The zeta potential of the clay suspensions was analyzed using a DELSA 440SX electrophoresis analyzer (Coulter, USA).

The interactions between anionic polymer and Ccy-clay were investigated by determining the isotherms of anionic polymer adsorption onto Ccy-clay particles in the absence of salt. The analysis was conducted in the same manner as described elsewhere [24]. Different amounts of 0.1 wt% Percol 173 or 0.1 wt% Percol E24, starting from 0.01 to 5 ml, were added to 5 ml of Ccy-clay dispersion obtained from the previous section. The mixture was further diluted with deionized and distilled water to a total volume of 10 ml. It was then mixed thoroughly and allowed to stand overnight at room temperature. The samples were then centrifuged at 3500 rpm for 15 min. The supernatant was separated and the amount of unadsorbed anionic polymer was determined by colloidal titration using Mutek PCD03 (Mutek, Germany).

#### 2.5. Analysis of clay flocs size

After the flocculation, the suspension containing clay flocs were collected in a 1000-ml beaker, and the size as well as the size distribution of the flocs was determined using an Analysette 22 COMPACT sizer (Fritsch, Germany). The arithmetic average of the particle size was determined by following the operating instructions.

#### 2.6. Determination of inclusion complex formation

To determine the efficiency of the cationic cyclodextrin in encapsulating model aromatic compounds during clay flocculation, the UV absorbance of the clay suspension containing either benzoic acid (BA) or toluene (as mimic soluble contaminants) before and after flocculation was analyzed. BA was added to the clay suspension to bring the initial concentration to about 200 ppm. The pH of the clay suspension was measured at about 3.5 and no electrolyte was

added. After 60 s of mixing, the cationic cyclodextrin and the anionic polymer were added in a predetermined basis. After PDA/DDJ flocculation, the clay flocs in water were collected in a 1000-ml beaker and allowed to settle. A quantity of 10 ml of the dispersion was taken from the sample and centrifuged at 3500 rpm for 15 min. The supernatant was then separated and analyzed for UV absorbance using a Spectronic 1001 Plus (Milton Roy Instruments, USA). The sample cell chamber was filled with the supernatant and UV absorbance was determined by following the standard operating procedure.

The same procedure was also employed when toluene was used instead of BA. When toluene was used the pH of the clay suspension was measured to be approximately 5.5. After 60 s of mixing, the cationic cyclodextrin and the anionic polymer were added on a predetermined basis.

To determine the amount of aromatic compound removed from the system, a calibration curve was prepared. The UV absorbance of several dilute aqueous solutions of the aromatic compounds was determined. The calibration curve was obtained from the absorbance at wavelengths 270 and 206 nm for the BA and toluene systems, respectively.

### 3. Results and discussion

#### 3.1. Characteristics of cationic-modified cyclodextrins

The zeta potential values of the cationic-modified cyclodextrins, prepared under various conditions, are presented in Table 1. Clearly, the unmodified cyclodextrin possessed a negative zeta potential ( $-23.9$  mV), with a charge density of about  $1.03 \times 10^{-6}$  eq/g. Upon being quaternized with ETMAC, the anionic properties of the unmodified cyclodextrin were evidently altered.

As can be seen from Fig. 1, a cationic cyclodextrin with the zeta potential value up to  $+19.7$  mV and the charge density around  $50.0 \times 10^{-6}$  eq/g was obtained as the cationic modification proceeded. From our experiments, it was also found that at a mole ratio (ETMAC:cyclodextrin) of about 3:1, the cationic groups already introduced charge reversal

Table 1  
Zeta potential of cationic-modified cyclodextrins with ETMAC

Cationic-modified cyclodextrin	Reaction pH	Reaction temperature (°C)	$\zeta$ potential (mV)
CECy-6	4.3	20	+17.1
CECy-7	8.3	20	+15.8
CECy-8	10.1	20	+18.1
CECy-9	11.4	20	+19.7
CECy-10	4.2	70	+14.6
CECy-11	8.4	70	+11.5
CECy-12	11.4	70	+16.0
Unmodified cyclodextrin	—	—	-23.9

Note. ETMAC: cyclodextrin mole ratio, 17.8:1; reaction time, 2 h.

on the cyclodextrin (zeta potential value of about +8.6 mV). The maximum zeta potential, close to +20.0 mV, was achieved at a higher ratio (17.8 mol/mol ratio). Both reaction temperature and reaction pH have limited influence on the substitution reactions, varying the zeta potentials from +11.5 up to +19.7 mV. Overall, moderate reaction conditions promote efficient introduction of the quaternary groups to cyclodextrin.

The  $^1\text{H}$  NMR spectrum of the cationic cyclodextrin, presented in Fig. 2, provides further evidence of quaternization. The structure of the product, i.e.,  $\beta$ -cyclodextrin-2-hydroxypropyltrimethylammonium chloride (HPTA- $\beta$ CD), is also shown. Several peaks were obtained that correspond to the protons in the attached alkyl chain of the functional group. The cleavage of the epoxide group led to peak (b) at about 5.2 ppm from the hydroxyl group OH in the alkyl chain. The other peaks were clearly distinguished as due to the protons in the alkyl chain. The peak intensity from the secondary hydroxyl groups OH<sub>2</sub> and OH<sub>3</sub> in cyclodextrin [25] did not change significantly, suggesting that substitution mainly occurred in the primary hydroxyl groups (OH<sub>6</sub> position), or at the narrow end of the torus.

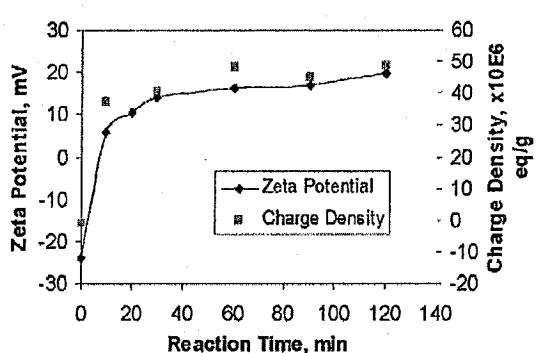


Fig. 1. Evolution of cationic characteristics of cyclodextrin (CECy-9) with time. ETMAC:cyclodextrin mole ratio, 17.8:1; reaction temperature, 20 °C; reaction pH, 11.4.

trin [25] did not change significantly, suggesting that substitution mainly occurred in the primary hydroxyl groups (OH<sub>6</sub> position), or at the narrow end of the torus.

### 3.2. Cationic cyclodextrin/anionic polymer systems for clay flocculation

#### 3.2.1. Effect of cyclodextrin or anionic polymer dosages

The relative turbidity of the clay suspension reflects the degree of flocculation. As can be seen from Fig. 3, the cationic cyclodextrin shows little effect on clay flocculation when used alone, unless the dosage of the cyclodextrin was increased up to 2.0% wt/wt on clay under such concentration that clay coagulation might occur. Interparticle bridging by the cationic cyclodextrin was apparently not adequate to induce agglomeration of clay particles.

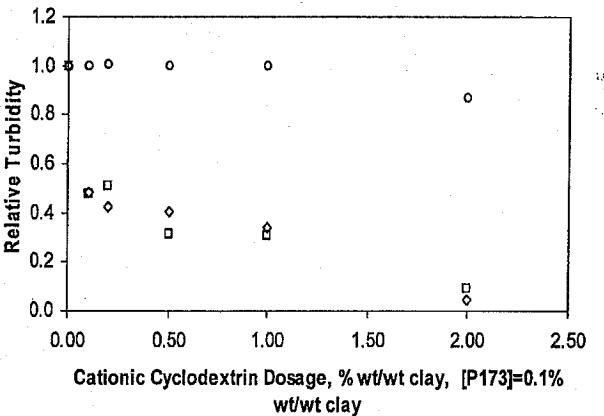


Fig. 3. Effect of cationic CD dosage on relative turbidity at constant P173 dosage. CECy-6 (□), CECy-9 (◇), CECy-6 alone (○).

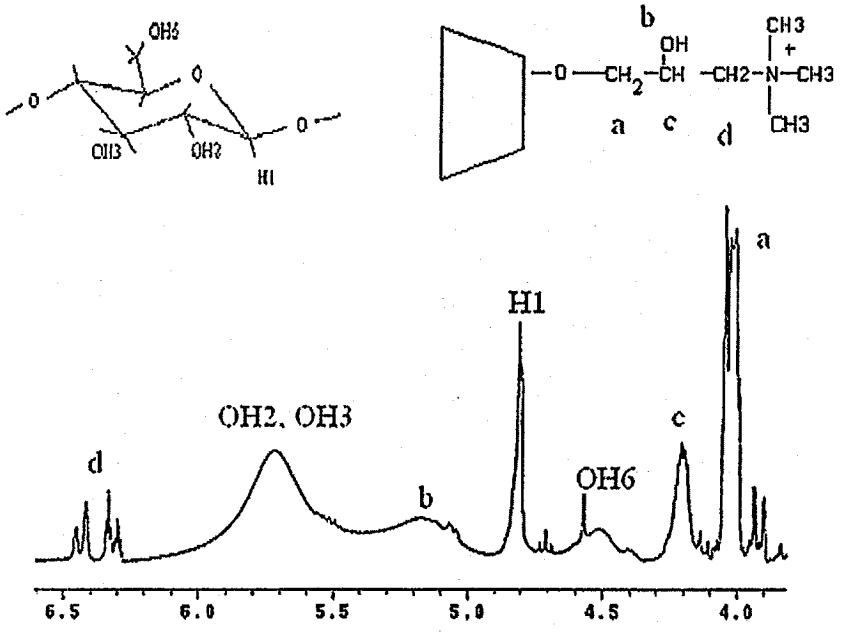


Fig. 2.  $^1\text{H}$  NMR spectrum of substituted cyclodextrin CECy-9.

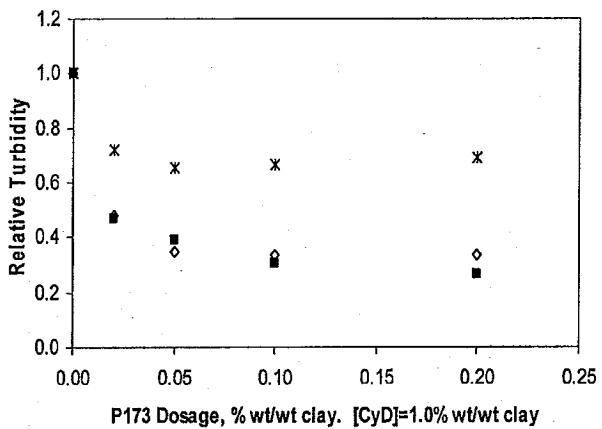


Fig. 4. Effect of P173 dosage on relative turbidity of clay suspension. Dosage of cationic CD maintained at 1.0% wt/wt clay. P173 alone (\*), CECy-6 (■), and CECy-8 (◇).

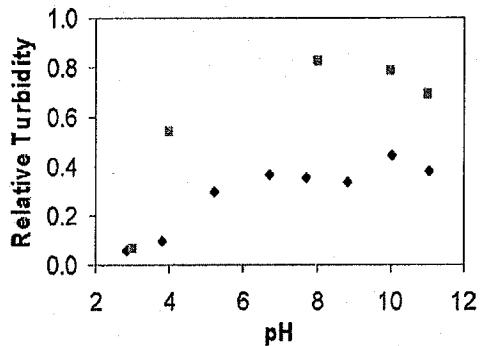


Fig. 5. Effect of clay suspension pH on flocculation efficiency. CECy-6/P173 (◆) and P173 alone (■).

When it was used in conjunction with the anionic polymer Percol 173, the flocculation of clay was significantly improved with increasing cationic cyclodextrin dosage. Both CECy-6 and CECy-9 samples performed effectively in reducing the relative turbidity of clay suspension. The same trend was observed at constant dosage of CECy-samples with increasing anionic polymer (P173) dosage (see Fig. 4). Both figures indicate the effectiveness of the dual-component system in improving clay flocculation. This implies strong interaction between the cationic cyclodextrin preadsorbed on clay surfaces and anionic polymer P173. Such an interaction also reduced the dosage of anionic polymer required for effective flocculation.

### 3.2.2. Effect of pH, ionic strength, and anionic polymer charge density

To further investigate the performance of cationic cyclodextrin, the pH of the clay suspension was adjusted, and the results are shown in Fig. 5. The performance of the anionic polymer alone was evidently dependent on pH. At low pH, the CECy-6/P173 system also showed dependency probably due to the anionic polymer. From neutral to basic conditions, the dual component system seemed to provide

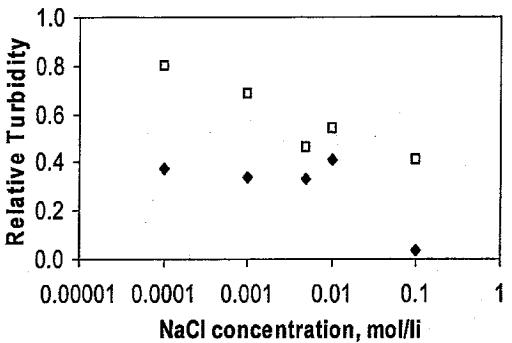


Fig. 6. Ionic strength effect on clay flocculation. CECy-6/P173 (◆) and P173 alone (□).

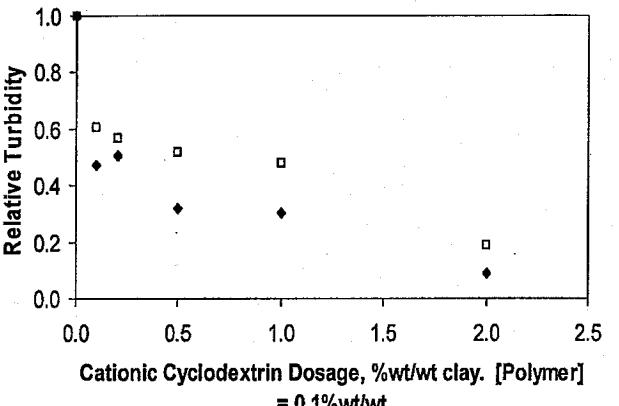


Fig. 7. Anionic polymer charge density effect on the relative turbidity of clay suspension as a function of the CECy-6 dosage. P173 (◆); PE24 (□).

a steady flocculation performance unaffected by increasing pH.

To determine the effect of electrolyte on clay flocculation various amounts of NaCl were added to the system. Results are presented in Fig. 6. Electrolytes steadily improved the flocculation efficiency of the anionic polymer-alone systems due to the coagulation effect. In contrast, no similar effect was observed for the dual-component system until the salt concentration was beyond 0.01 M.

To evaluate the effect of the charge density of linear polymers on the flocculation efficiency, an anionic polymer with higher charge density, Percol E24, was used. The molecular weight of E24 is similar to that of P173. Results, as shown in Fig. 7, indicate that E24 was less effective in inducing clay flocculation than P173. This observation is in agreement with the previous results obtained from clay flocculation performed by Ovenden et al. [22]. However, the interaction between Percol E24 and cationic cyclodextrin is still strong, reducing the relative turbidity of clay suspension effectively.

### 3.2.3. Adsorption isotherms of anionic polymers on pretreated clay particles

The interaction between the cationic-modified cyclodextrin and anionic polymer determines effective flocculation. Adsorption of cationic cyclodextrin on clay particles has

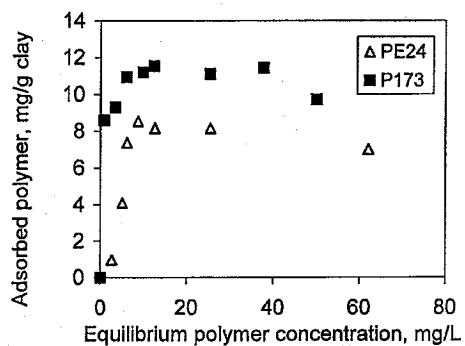


Fig. 8. Adsorption isotherm of anionic polymer/clay system at pH 4.5. Clay particles pretreated with CECy-6. [Clay] = 2.5 g/L; [CECy-6] = 0.5% wt/wt clay.

been confirmed from zeta-potential measurements of the mixture of clay with cationic cyclodextrin. Results indicate that charge neutralization was reached at 0.5–1.0% of CECy wt/wt on clay. To utilize such CECy pretreated clay particles as substrates, the interaction between anionic polymers and cationic cyclodextrin was also investigated. Results are presented in Fig. 8.

As can be seen, the amount of polymer adsorbed at the saturation point was 11.5 mg/g for Percol 173, whereas a lower adsorbed amount (8.1 mg/g) was found for Percol E24. The different adsorption behavior is attributed to the fact that the anionic polymer with high charge density (Percol E24) tends to flatten on the surface of the clay particles after adsorption and electrostatic repulsion occurs between anionic polymer chains during the adsorption. The anionic polymer with low charge density (Percol 173) achieved high adsorption with extended tails and loops, facilitating the flocculation processes.

Apparently, the amount of polymer adsorbed was higher than that obtained if the clay particles were not pretreated with cationic cyclodextrin. The saturation values from Fig. 8 are consistent with the findings obtained from our previous work, where the amount of anionic polymer adsorbed onto untreated clay particles was 6.7 and 5.2 mg/g for Percol 173 and Percol E24, respectively [23]. Overall, the relatively poor interaction between Percol E24 and clay particles, regardless of their being pretreated with cationic cyclodextrin or not, led to relatively ineffective flocculation of filler particles.

To reveal the floc characteristics, the average size of clay flocs after flocculation was determined, and results are presented in Table 2. Clay particles in water suspension have an average unflocculated particle size of about 2  $\mu\text{m}$ . When the anionic polymer was added to the suspension, agglomeration of clay particles was observed, giving an average floc size of about 9.7  $\mu\text{m}$ . With the addition of cationic cyclodextrin to the system, the flocculation of clay particles improved significantly, showing an increase in floc size, corresponding to the reduction in the relative turbidity as the dosage of cationic cyclodextrin increased (see Fig. 3). The largest flocs, with an average of 35.2  $\mu\text{m}$ , occurred when the cationic

Table 2

The sizes of clay flocs formed in dynamic flocculation using a dual-component system, CECy-6/P173

CECy-6 to P173 ratio (wt:wt)	Arithmetic average ( $\mu\text{m}$ )	SD <sup>a</sup> ( $\mu\text{m}$ )
20:1	14.0	3.7
10:1	21.4	4.6
5:1	35.2	5.9
2:1	25.8	5.1
1:1	19.4	4.4
0:1	9.7	2.1

<sup>a</sup> SD: standard deviation.

cyclodextrin-to-P173 dosage ratio was 5:1. Apparently, the bridging of clay particles by the anionic polymer through the interaction of the cationic cyclodextrin onto the surface of clay particles led to the formation of clay flocs, reducing the relative turbidity of the suspension and increasing the floc size. Further increases in the amount of cationic cyclodextrin in the suspension, while maintaining the polymer amount added to the clay suspension, eventually produced smaller clay flocs. This could be attributed to chain reconfiguration of the adsorbed polymer or complex formation between free cyclodextrin in aqueous solution and anionic polymers.

### 3.3. Simultaneous removal of dissolved and colloidal substances via formation of inclusion complex and flocculation

#### 3.3.1. Sorption behavior of cationic cyclodextrins toward aromatic compounds

As a potential novel approach to remove dissolved and colloidal substances simultaneously, an attempt has been made to entrap the model hydrophobic species, i.e., benzoic acid and toluene in the clay suspension, via inclusion complex formation with the modified cyclodextrin nanospheres, followed by flocculation in conjunction with anionic polymer. While inducing clay flocculation along with anionic polymer, the modified cyclodextrin still has a hydrophobic cavity capable of forming inclusion complexes with organic specimens in the system. To further investigate the capability of the modified cyclodextrin to encapsulate aromatic compounds during flocculation, benzoic acid and toluene were added to the filler suspension (at a concentration of about 200 ppm) prior to addition of the flocculants.

Fig. 9 shows a typical spectrum of UV absorbance for the supernatant containing toluene clay, cationic cyclodextrin, and anionic polymer. The corresponding concentrations of benzoic acid in the supernatant under various conditions are also presented in Table 3. Similarly, Table 4 presents the results obtained from the system containing toluene as a mimic aromatic contaminant. As can be seen from Table 3 for the BA system, filler clay itself has affinity toward benzoic acid due to its layered structure, removing about 12% BA in the absence of flocculants. Cationic cyclodextrin (CECy-6) alone entrapped approximately 7% of BA.

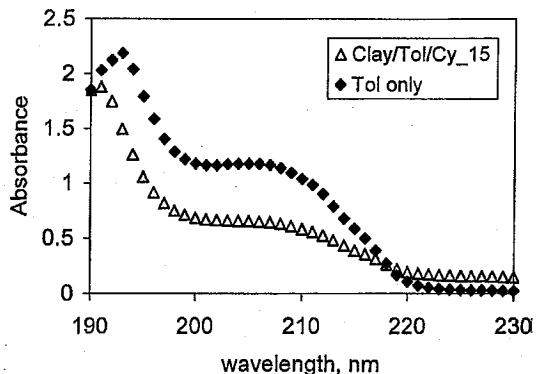


Fig. 9. UV absorbance for system containing 200 ppm toluene only (◆) and clay suspension supernatant after addition of 1.5% wt/wt clay CECy-6 and 0.1% wt/wt P173 (△).

Table 3  
Absorbance of benzoic acid (BA) with the equivalent concentration in the supernatant after flocculation

	Absorbance at 270 nm	Concentration (ppm)	Removal (%)
BA only	1.42	200	0
Clay + BA	1.24	175	12
Clay + BA + P173	1.09	154	23
Clay + BA + CECy-6 + P173	1.01	142	29
BA + CECy-6 + Clay + P173	1.02	145	28
BA + CECy-6	1.32	186	7

Note. [CECy-6] = 1.5% wt/wt filler; [P173] = 0.1% wt/wt filler.

It should be noted that the dosage of cyclodextrin is only 1.5% on clay. At the same dosage, the amount of absorption by cyclodextrin would be expected substantially higher. BA removal was further increased, up to 29%, as anionic polymer was added. Anionic polymer, along with clay particles, reduced the amount of BA to about 23%. Clearly, the difference was close to the amount removed by the inclusion of BA in the hydrophobic cavity of the cyclodextrin. Apparently, clay flocs formed by anionic polymer are also effective in absorbing BA. However, the mechanism behind this has not been fully understood.

Much better inclusion induced by cationic cyclodextrin was observed in toluene system; whereas clay itself has little effect on the removal of toluene. As shown in Table 4, up to 22% of toluene was removed by the cyclodextrin. As the addition of anionic polymer, the total removal was increased up to 45%. This suggests that the modified cyclodextrin was indeed capable of including aromatic compounds from the suspension. Addition of the anionic polymer alone reduced the amount of toluene in the suspension to about 24%, which might also be attributed to the absorption enhanced by clay flocs.

Overall, the results of UV absorbance demonstrate that cationic cyclodextrin is effective in lowering mimetic aromatic contaminants via inclusion complex formation. Although both aromatic compounds have one benzene ring that could fit inside the small 7.80-Å cavity of the modified  $\beta$ -cyclodextrin nanosphere, less benzoic acid was removed

Table 4

Absorbance of toluene (Tol) with the equivalent concentration in the supernatant after flocculation

	Absorbance at 206 nm	Concentration (ppm)	Removal (%)
Tol only	1.18	200	0
Clay + Tol	1.19	202	0
Clay + Tol + P173	0.89	152	24
Clay + Tol + CECy-6 + P173	0.65	110	45
Tol + CECy-6 + clay + P173	0.69	117	42
Tol + CECy-6	0.94	156	22

Note. [CECy-6] = 1.5% wt/wt filler; [P173] = 0.1% wt/wt filler.

Table 5

Effect of benzoic acid (BA) and toluene on clay suspension relative turbidity and floc size at constant CECy-6 and P173 dosage of 1.5% wt/wt clay and 0.1% wt/wt clay, respectively

BA (ppm)	Toluene (ppm)	Relative turbidity	Floc size ( $\mu\text{m}$ )
200	0	0.12	11.9
0	0	0.13	17.6
0	200	0.13	15.0

through formation of inclusion complex than toluene. This result is in agreement with the literature, whereby the water-soluble benzoic acid is bound weakly in the cyclodextrin cavity, while the nonpolar toluene is easily complexed [3]. Moreover, the addition order had little effect on the removal of aromatic compound, regardless of cationic cyclodextrin being mixed with the aromatic compounds before or after the addition of filler clay. This suggests that filler clay particles themselves do not contribute to the entrapment of toluene; however, the clay flocs did. Whether the removal of toluene in anionic polymer/clay systems was attributed to the clay flocs or polymer coils is still a subject under debate.

### 3.3.2. Effect of the inclusion on clay flocculation

Results above clearly indicate the effectiveness of cationic-modified cyclodextrin in encapsulating the model hydrophobic compounds via inclusion complexation. The question raised is whether the modified cyclodextrin with included guest compound has a negative impact on clay flocculation. To identify such an effect, the results of clay flocculation induced by P173 and CECy-6 including either toluene or benzoic acid are presented in Table 5. The results for the control sample, as well as the floc sizes, are also shown. A reduced clay floc size was observed for the flocculation systems involving modified cyclodextrins with included guests, whereas the excellent flocculation efficiency (i.e., low relative turbidity) was remained. Fig. 10 illustrates a schematic of such a simultaneous separation process via cyclodextrin sorption and the bridging between clay platelets induced by cationic cyclodextrin/anionic polymer association. To elucidate why the floc size was reduced slightly, it is necessary to assess the floc strength or the effect of free model aromatic compounds on interparticle bonding, as well as the potential reformation of polymer chains, which might influence the electrostatic association between with anionic polymer

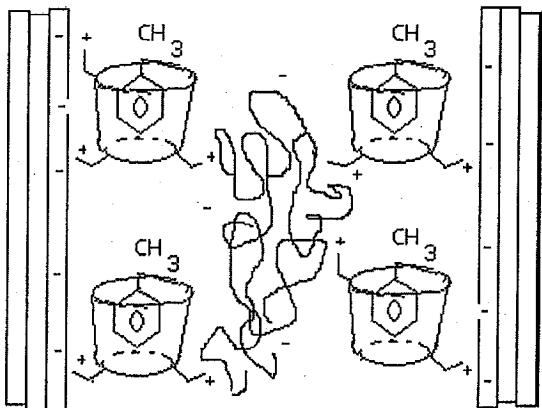


Fig. 10. A schematic of the toluene included in cationic  $\beta$ -cyclodextrin and the bridging induced by anionic polymer between two clay platelets.

and encapsulated cyclodextrin nanospheres or clay particles. Such work is still ongoing.

#### 4. Conclusions

The key conclusions from this work are as follows:

- Quaternization of  $\beta$ -cyclodextrin was achieved using the cationic agent EMATC. The cationic characteristics of the modified cyclodextrin were confirmed by NMR and electrophoresis measurements.
- The modified cyclodextrin, in conjunction with a linear anionic polymer, consisted of a novel nanoparticles flocculation system. Moreover, such a dual-component system provided a potential approach for the simultaneous removal of dissolved and colloidal substances, facilitating the separation processes for various applications.
- Effective clay flocculation was obtained at a ratio of cationic cyclodextrin:anionic polymer equal to 5:1 or higher. The presence of cationic cyclodextrin also reduced the amount of anionic polymer (0.1 wt% on clay or lower) for the effective flocculation.
- The dual-component flocculation system was less sensitive to pH, compared with anionic polymer alone system, whereas the anionic polymer with higher charge density led to less effective flocculation, due to the weak adsorption of such polymers on both the clay particles pretreated with cationic cyclodextrin and the untreated clay particles.

- The cationic charges present in the modified cyclodextrin contributed to charge reduction on the surface of clay particles, allowing more efficient interparticle bridging by the anionic polymer chains. The bridging of clay particles was the likely mechanism being supported in this work.
- As a nanospherical absorbent, the cationic-modified cyclodextrin was indeed capable of encapsulating aromatic compounds via formation of inclusion complex. The modified cyclodextrin showed a strong affinity toward toluene, and meanwhile maintained flocculation ability, after having included the guest molecules.

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**(10) RELATED PROCEEDINGS APPENDIX**

As the appellants are not aware of any other related proceedings, no copies of decisions rendered by a court or the board are attached.